## The salt-tolerant rice selection suitable for saline waste water irrigation in Ergene Basin

# Mehmet AYBEKE, Hande DEMİRAL

Trakya University, Faculty of Science, Dept. of Biology, Balkan Campus, 22030, Edirne,

# TURKEY.

## \*: For correspondence. Mehmet Aybeke

Trakya University, Faculty of Science, Dept. of Biology, Balkan Campus, 22030, Edirne,

TURKEY.

E-mail; mehmetaybeke@yahoo.com

GSM: +90 5062931789, Office Tel: +90 284 2352824-25-26 (1183)

#### Abstract:

The aim of this study is to use morphological and physiological parameters to select both salt tolerant and high yielding rice cultivars convenient for growth in the salinity conditions of the Ergene Basin. Accordingly, the panicle fertility and 1000-grain weight were determined in 40 rice cultivars, and a cation analysis (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>) in the roots was performed. The results of the panicle fertility data classified the following eight cultivars as "highly fertile": Kral, Kırkpınar, Sürek-95, 7721, Kros-424, Gala, Veneria, and Durağan. On the basis of the 1000grain weight, the following eight cultivars measured over 20 g: Kıral, Kırkpınar, Kros-424, Osmancık, Durağan, Halilbey, Kargı, and Rodina. In both the panicle fertility and the 1000grain weight, higher values are seen in the cultivars Durağan, Kros-424, Kırkpınar and Kıral, with Kırkpınar and Kıral demonstrating the highest values. According to the cation analysis in the roots, the Na<sup>+</sup> values are generally higher than the K<sup>+</sup> values, but lower than the Ca<sup>2+</sup> values. In terms of Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na-K selectivity, the Na-Ca selectivity ratios of the four highest-yielding cultivars (Durağan, Kros-424, Kırkpınar and Kıral) were similar to each other and at the middle-level according to the other "fertile" or "low fertile" cultivars. The extreme values of these parameters lead to yield loss. Consequently, the Durağan, Kros-424, Kıral and especially Kırkpınar cultivars are the most salt-tolerant and highest yielding rice cultivars for use in the Ergene Basin.

Keywords: salt stress, Na salinity, rice, Oryza, screening, Ergene

### **1. Introduction**

The Ergene basin contains the most important agricultural fields in Turkey, as well as a high potential for industry. The basin's major source of income is rice agriculture. In the Ergene basin, rice is cultivated on approximately 13 222 ha, and its harvest accounts for 30% of the total rice production in Turkey (Anonymous, 2008). Thus, maintaining a supply of suitable water to meet the agricultural aims is a very important problem. The Ergene River, which is 194 km in length and contains a drainage area of 10 730 km<sup>2</sup>, provides most of the water in the basin. The greatest problem for both the Ergene Basin and the Ergene River is the water salinity stemming from leather factories. Thus, salinity is the major environmental factor limiting rice growth and productivity in the basin. The detrimental effects of high salinity on plants, such as plant death and/or decreases in productivity, can be observed at the wholeplant level. Many plants develop mechanisms to either exclude salt from their cells or to tolerate its presence within the cells. This requires a major focus on improving the productivity and sustainability of rice-based farming systems. Thus, the selection and breeding of salt-tolerant cultivars that can withstand high levels of such stresses, and that can be grown without costly soil amendments, are of paramount importance. The screening and breeding of rice varieties for tolerance to salinity or alkalinity have been carried out for over three decades (Yeo et al. 1990, Flowers 2004), and various methodologies have been used (Flowers and Yeo 1981, Bal et al. 1986, Qadar 1988, Rao et al., 2008) to screen out tolerant varieties. In particular, morphological parameters, such as panicle fertility and 1000-grain weight, and physiological characteristics, such as cation analysis in the roots, were successfully used in tests for the selection of salt-tolerant rice cultivars (Anonymous, 2003; Khan and Abdullah 2003; Zeng 2005; Yakıt and Tuna, 2006; Rao et al., 2008; Sepaskhah and Yousofi-Falakdehi, 2009; Murtaza et al., 2009). Therefore, the aim of this study is to select

both salt tolerant and high yielding rice cultivars according to various morphological and physiological parameters.

### 2. Materials and Methods

The rice grains (Oryza sativa L.) for the field experiments were obtained from the Trakya Agricultural Research Institute, and the list of 40 rice cultivars is given in Table 1. The experiments were conducted for two years, 2009 and 2010, in the Ergene Basin. The experimental area is located at 41° 8' and 45N 26° 34' 47E in the sub-humid, sub-tropical Mediterranean region. The rice grains were sown in a field in Salarlı village, Uzunköprü-Edirne-Turkey, which is located in the middle of the Ergene basin. The experiment was replicated at three different sites using a randomised complete block design. The plot size of one site was  $13x17 \text{ m}^2$ . All sites had dense saline soil. The distances between the plants and between the rows were 20 cm each. The N:P:K fertiliser was applied as urea, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O at the rate of 8:7:5 g m<sup>-2</sup> and ZnSO<sub>4</sub> 1 g m<sup>-2</sup> (Khan and Abdullah, 2003). The temperature during the day ranged from 21 to 31 °C, whereas the temperature at night fluctuated between 25 and 32 °C. The humidity ranged from 50 to 75% during the growing period. The water of the Ergene River was used for irrigation. The soil and water analysis of the Ergene basin is as follows: Soil pH: 7,47 (light alkali); 1.591,00 mmhos/cm; Water pH: 7,96; ECx10<sup>8</sup>: 3580 micromhos/cm; SAR: 18.71; and irrigation water class: C4S3. In the present study, the spikelet fertilities and 1000-grain weight were investigated, and a cation analysis in the roots  $(Na^{+1}, K^{+1}, Ca^{+2})$  was performed. For these cultivars, the fertile florets per panicle and the spikelet sterility percentage at the maximum flowering stage were recorded. The 1000 grains for each sample were selected from a random sample, and they were counted using an electronic seed counter and weighed. The morphometric data for each variety, such as the

fertile florets per panicle and the grain yield per plant, were recorded after maturity using three plants from each replicated plot (Rao et al., 2008). Significant levels were taken at P<0.05 and P<0.01. Statistical analyses of the data were performed with MINITAB software (Mini Inc.PA). The concentrations of Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> in the roots were determined on nitric-perchloric acid digests using inductively coupled plasma optical emission spectrometry for the Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ratios in the samples (ICP atomic emission spectrometer, Perkin-Elmer Co., Norwalk, CT, USA). The K, Na and Ca content represent the concentrations (mg kg<sup>-1</sup> dry wt) in the roots.

The K-Na selectivity was calculated using the equation described by Pitman (1976):

 $S_{K,Na} = (K \ content/[K] \ medium)/ \ (Na \ content/[Na] \ medium),$ 

where  $S_{K,Na}$  represents K-Na selectivity; K content and Na content represent the concentrations.

Na-Ca selectivity ( $S_{Na,Ca}$ ) was calculated using the Gapon selectivity constant (Kg) (Sposito, 1981; Zeng, 2005)

$$Kg = (E*Na(A_{Ca})0.5)/(E_{Ca*}A_{Na}),$$

where E represents the equivalent fraction of a given cation, and A represents the activity of the ion in solution. Thus, Kg relates the equivalent fractions of the exchange ions to the activities of the ions in solution (Zeng, 2005).

#### 3. Results

All morphometric, physiological and statistical data were given in Table 1. On the basis of the panicle fertility ratios, the Kral, Kırkpınar, Sürek-95, 7721, Kros-424, Gala, Veneria, and

Durağan cultivars are considered "highly fertile". The Ece, Osmancık, Ergene, N-41-T-OT, Koral, Altınyazı, Halilbey, and Tunca cultivars have been confirmed to be "fertile". The rest of the cultivars are designated "partially fertile". The 1000-grain weight measurements for the Kıral, Kırkpınar, Kros-424, Osmancık, Durağan, Halilbey, Kargı, and Rodina cultivars are all over 20 g (Table 1). All of the differences among these morphometric data are statistically significant. The cultivars that exhibit both high fertility and a high 1000-grain weight are Durağan, Kros-424, Kırkpınar and Kıral (Table 1). The values for the Kral and Kırkpınar cultivars are especially greater than the values for the other cultivars. For all investigated cultivars, the Na<sup>+</sup> ionic concentration are higher than the K<sup>+</sup> concentration. The Ca<sup>2+</sup> values are higher than the  $Na^{+1}$  and  $K^+$  values in all the cultivars, with the exceptions of the Koral, Beser, Karacadağ, Akceltik and Ribe cultivars. The  $Na^{+1}/K^{+1}$  value varies between 1,2 (min) and 5,4 (max), depending on the cultivar. The highest and the lowest  $Na^+/K^+$  values are in the Ribe (5,4) and Serhat (1,2) cultivars, respectively, in which the fertility and 1000-grain weight are also lower (Table 1). In cultivars with "high fertility", such as Kral, Kırkpınar, Sürek-95, 7721, Kros-424, Gala, Veneria, and Durağan, the  $Na^+/K^+$  values vary between 2 (min) and 3,1 (max) depending on the cultivar. The  $Na^+/K^+$  values of the Kral, Kırkpınar, Durağan and Kros-424 cultivars (all of which are considered "highly fertile" and have high 1000-grain weights) are 3.10, 2.36 2.33 and 2.52, respectively. In particular, the  $K^{+1}$  levels in the Kırkpınar, Durağan and Kros-424 cultivars are greater than those of Kral. The Na<sup>+</sup>/Ca<sup>2+</sup> data in these cultivars vary between 0.39 and 1.9. The highest and the lowest  $Na^+/Ca^{2+}$  values were found in Ribe (1,9) and N-1-41-T-OT (0,39), in which both the fertility and the 1000-grain weight are also low. Similar findings were made for Aromatic ( $Na^{1+}/Ca^{2+}=0.43$ ) and the Karadeniz, Ranballi, Beşer, Koral and Karacadağ cultivars, all of which had values of Na<sup>+</sup>/Ca<sup>2+</sup> over 1. In the Durağan, Kros-424, Kral and Kırkpınar cultivars, which are all highly fertile and have high 1000-grain weights, these parameters range between 0,72 and 0,86.

According to the K-Na selectivity, the lowest and the highest data are found in Ribe (0.34) and Serhat (1,53). The fertility and 1000-grain weight of these two cultivars are low (Table 1). In "fertile" cultivars such as Osmancık, Ece, Ergene, N-1-41-T-OT, Altınyazı, Halilbey, and Tunca, the selectivity value ranges from 0,69 to 1,07. In particular, the 1000-grain weight is very low in N-1-41-T-OT, although its selectivity is 1,3. For Kral, Kros-424, Kırkpınar and Durağan, (higher fertile and higher 1000-grain weight) these values are 0,59, 0,73, 0,78, and 0.79, respectively (Table 1). The K selectivity is especially greater in Kirkpinar and Durağan. The lowest value for Ca-Na selectivity is found in Plovdiv (2,01), whereas the highest value is in Ribe (9,65). In both of these cultivars, the fertility and 1000-grain weight are very low. In the "fertile" cultivars, Ece, Osmancık, Ergene, N-41-T-OT, Koral, Altınyazı and Halilbey, the values range from 3 to 5,61. Regarding the "highly fertile" cultivars, Kral, Kırkpınar, Sürek-95, 7721, Kros-424, Gala, Veneria, and Durağan, the values are between 2,98 and 6,45. In the Kral, Kros-424, Kırkpınar and Durağan cultivars, which are all highly fertile and have high 1000-grain weights, these values are 2,98, 3,77, 4,72, and 5,45, respectively. The Ca selectivity of Kırkpınar, which indicates the highest 1000-grain weight value, falls between the values found in Kral and Durağan.

Cultivar	Na*	K*	Ca*	Na/K	Na/Ca	S <sub>Na,K</sub> <sup>a</sup>	S <sub>Na,Ca</sub> b	Fert. <sup>c</sup>	Fert.C <sup>d</sup>	1000-grain weight <sup>e</sup>
Kıral	6396	2058	7432,67	3,10	0,86	0,595	2,98	98	high fertile	21,6 jkl
Kırkpınar	7867,31	3331,97	9565,82	2,36	0,82	0,784	4,72	98	high fertile	24,0 opq
Sürek-95	9455,79	3521,25	10871,28	2,68	0,86	0,689	6,45	91.6	high fertile	13,6 lmno
7721	5814,83	2953,32	9181,98	1,96	0,63	0,94	3,35	93.9	high fertile	12,7 nop
Ece	5587,11	3231,42	9160,22	1,72	0,60	1,07	3,21	88.8	fertile	19,8 no
Kros-424	7144,25	2826,97	8416,47	2,52	0,84	0,732	3,77	91	high fertile	20,0 cde
Gala	8487,88	3356,54	9723,44	2,52	0,87	0,732	5,17	93.9	high fertile	15,0 klm
Veneria	6615	2716	8549,08	2,43	0,77	0,76	3,54	96	high fertile	9,3 s
Osmancık	7720,73	2911,31	9349,7	2,65	0,82	0,698	4,5	85	fertile	21,3 bc
Ergene	7012	2845	9212	2,46	0,76	0,751	4,05	88	fertile	19,6 nopq
N-41-T	4368,19	3081,8	10987,08	1,41	0,39	1,306	3,01	87.8	fertile	11,0 qr
Durağan	7967,46	3408	10919,54	2,33	0,72	0,792	5,45	91.7	high fertile	21,3 hij
Koral	12000,82	3198,24	7450,17	3,75	1,61	0,493	5,61	87.2	fertile	15,0 klm

**Table 1:** All morphometric and phisyological data of investigated 40 rice cultivars

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Altınyazı	7931,98	3247,75	9146,42	2,44	0,86	0,758	4,55	84.5	Fertile	10,3 rs
Halilbey	5870,41	2967,13	8711,71	1,97	0,67	0,935	3,20	81.6	fertile	21,2 efg
Neğiş	6433,25	2219,8	9470,47	2,89	0,67	0,639	3,82	62	partial fertile	15,7 jkl
Trakya	6107,32	2518,49	7394,01	2,42	0,82	0,763	2,83	68	partial fertile	17,7 ghı
Demir	5012	2513	7058	1,99	0,71	0,928	2,21	53	partial fertile	16,3 ıjk
Şumnu	7303	2531,75	8212,54	2,88	0,88	0,642	3,76	74	partial fertile	16,7 hıj
Gönen	7324,63	3298	9754,43	2,22	0,75	0,833	4,48	71	partial fertile	11,3 pqr
Kızıltan	5088,78	2803,23	9432,65	1,81	0,53	1,019	3,01	69	partial fertile	13,7 mn
Beşer	9215	2831	6258	3,25	1,47	0,569	3,61	74.9	partial fertile	13,0 no
Kargı	5805	2139,07	9751,54	2,71	0,59	0,682	3,55	60.7	partial fertile	22,3 b
Edirne	6529,71	2178,29	7873,79	2,99	0,82	0,617	3,22	63	partial fertile	17,7 ghı
Aromatik	4258	2896	9863	1,47	0,43	1,259	2,63	56.7	partial fertile	12,0 pqr
Karadeniz	9142,91	2929,08	6511,53	3,12	1,40	0,593	3,73	72	partial fertile	19,3 def
Rocca	6889,07	2789,29	8463,83	2,46	0,81	0,749	3,65	72	partial fertile	15,0 klm
Yavuz	7614,68	3031,93	8501,71	2,51	0,89	0,737	4,06	74	partial fertile	16,3 ıjk
Maratelli	5201	2453	6257	2,12	0,83	0,873	2,04	59	partial fertile	15,0 klm

Ranbelli	6321	3621	5201	1,74	1,21	1,06	2,06	72	partial fertile	17,0 hıj
Meriç	8141,98	2982,23	9883,94	2,73	0,82	0,678	5,04	73	partial fertile	18,0 fgh
Plovdiv	4126,63	2417,86	7774,22	1,70	0,53	1,084	2,01	64	partial fertile	15,0 klm
Serhat	2541	2105	5214	1,20	0,48	1,533	0,83	54	partial fertile	10,3 rs
Karacadağ	5873	3012	3528	1,94	1,66	0,949	1,30	77.6	partial fertile	10,6rs
Diyarbakır	3521	2015	3528	1,74	0,99	1,059	0,77	71	partial fertile	17,7 ghi
Akçeltik	8143,14	3024,3	8069,37	2,69	1,00	0,687	4,12	54	partial fertile	11,3 pqr
Tunca	6887,46	3104,4	8882,7	2,21	0,77	0,834	3,83	79.9	fertile	15,7 jkl
Ribe	17098,8	3163,24	8994,43	5,40	1,90	0,342	9,65	63	partial fertile	12,3 pqr
İpsala	7377,64	3046,57	7361,37	2,42	1,00	0,764	3,40	73.9	partial fertile	16,3 ıjk
Sarıçeltik	7235	2963	7985	2,44	0,90	0,758	3,62	62	partial fertile	9,3 s
Rodina	6251	3201	6847	1,95	0,91	0,948	2,68	54	partial fertile	20,3 cd

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\*: mg/kg

a: K-Na selectivity

b<sup>i</sup> Ca-Na selectivity

c: Fert: Fertility

d: Fert.C: Fertility cluster (according to Anonymus, 2003)

e: 1000-grain weight followed by the same letter are not significantly different at 5% level

#### 4. Discussion

In this study, through an analysis of select morphometric and physiological parameters, the salt-tolerant and high yielding rice cultivars best suited to the Ergene Basin, where 30% of Turkey's rice production is met, have been determined. From an analysis of panicle fertility, the following eight cultivars are classified as "highly fertile": Kral, Kırkpınar, Sürek-95, 7721, Kros-424, Gala, Veneria, and Durağan. The cultivar Osmancık, which is the most widely grown cultivar in the Basin, is classified as "fertile". The following eight cultivars exhibited 1000-grain weight measurements over 20 g: Kıral, Kırkpınar, Kros-424, Osmancık, Durağan, Halilbey, Kargı, and Rodina. From an evaluation of both the panicle fertility and the 1000-grain weight, the highest values are seen in four cultivars: Durağan, Kros-424, Kırkpınar and Kıral (Table 1). The values for Kırkpınar and Kıral are especially high. According to a cation analysis in the roots, the Na values are generally higher than the K values, but lower than the Ca values, which is consistent with a recent study (Zing, 2005). According to the Na/K ratio, the extreme values were 1,2 and 5,4. However, these values are moderate in Kros-424, Durağan Kıral, and Kırkpınar, all of which were deemed "highly fertile" and high 1000grain weight cultivars. Notably, changes in the  $K^+$  in the Kros-424, Durağan and Kırkpınar cultivars were greater than those in Kral. This finding indicates that K<sup>+</sup> in these cultivars is used excessively against salt stress. Additionally, the Na-K selectivity values of these cultivars (Kros-424, Kırkpınar, Durağan) are similar to each other and at a middle-level. Similar findings appeared in the  $Na^+/Ca^{2+}$  and Na-Ca selectivity ratios. The extreme values were 0.39-1.9 (min, max) for Na<sup>+</sup>/Ca<sup>2+</sup> and 2.01-9.65 (min, max) for Na-Ca selectivity. These values in Kros-424, Durağan, Kıral and Kırkpınar, cultivars that are considered "highly fertile" and have high 1000-grain weights, were clearly at the middle levels. In brief, both the panicle fertility and the 1000-grain weight indicate a reduction in all physiological data at the extreme points. In our view, these excessive fluctuations in K and Ca values negatively affected the plant's tolerance to salt stress; in other words, these fluctuations reduced the plant's yield. According to the available literature, K<sup>+</sup> plays an active role in protein synthesis and in ensuring the link between tRNA and ribosomes in cytoplasm. When the concentration of K<sup>+</sup> in the cytoplasm is greater than 180 mM, it interrupts protein synthesis and caused a breakdown in the polysomes. In general, K<sup>+</sup> is has a lesser impact as an inhibitor than Na<sup>+</sup>. In brief, although the amount of  $K^+$  plays a key role in protein metabolism, the abnormal increase of the K+ quantity while altering NA salinity leads to a toxic effect on the plant. In previous studies of this issue, the amelioration of Na+ toxicity was achieved by Ca+2 (Khan 2001; Soussi et al., 2001; Munns et al. 2002; Umno et al., 2002; Aslam et al. 2003). Ca<sup>+2</sup> play an important role in the protection and sustainability of the plant membrane structure, permeability of the cell wall, wall enzymes, and ion transport. A low  $Na^+$  / excessively high Ca<sup>+2</sup> ratios caused morphological anatomical defects in the developmental stage of plants (Cramer 1992; Aslam et al. 2003). In this case, accumulation of both Na<sup>+</sup> and K<sup>+</sup> occurred in the vacuole rather than in the cytosol (Devienne et al., 1994; Bell et al., 1995; Fricke et al., 1996; Leigh 1997; Paridaa et al., 2005; Flowers and Colmer 2008); when this accumulation occurs, the cytoplasmic and apoplastic concentrations of Na+ are maintained at low levels, approximately 10-30 mM (Carden et al. 2003; Tester & Davenport 2003), or the toxic ion absorption is restricted at the root level. Thus, the Na+ streaming from root to shoot will be limited, and salt stress will be decreased at the whole plant level due to the reduced apoplastic flow, which is a significant pathway for Na<sup>+</sup> uptake in rice under saline conditions (Carden et al. 2003; Tester & Davenport 2003; Veena et al., 2005; Flowers and Colmer 2008; Faiyue et al., 2011).

In salinity, the most common ion is the Na<sup>+</sup>. Soils bearing a high Na<sup>+</sup> level are toxic for plants. As demonstrated in several previous studies, this toxicity occurs because an excessive salt concentration has a negative impact on the ionic balance, water uptake and volume, photosynthesis, respiration, and protein synthesis, and it disturbs the nucleic acid metabolism, enzyme activity and other metabolic processes (Blumwald et al., 2000; Sairam et al., 2002; Munns et al., 2006; Giri et al., 2007; Chaves et al., 2008; Takahashi et al., 2008). As several studies have noted, when considering both Na+ salinity and the excessive fluctuations in K<sup>+</sup> and Ca  $^{2+}$  levels for the alteration of stress, it is logically inferred that optimal Na $^+/K^+$  and Na<sup>+</sup>/Ca<sup>+2</sup> ratios are important determinants of salt tolerance (De Lacerda et al., 2005; Raja Babu et al., 2005; Roshandel & Flowers, 2009). Similar studies have been performed in different plants. For example, Hajibagheri et al. (1989) found that  $Na^+/K^+$  ratios were 1.88 and 3,91 in the roots of two corn cultivars and that the cultivar with the 3,91 ratio had a greater vacuolar compartmentalisation of Na<sup>+</sup> than the other cultivar. In wheat, the salinity tolerance is related to a decrease in the  $Na^+/K^+$  ratio (Dvorak et al., 1994; Gorham et al., 1997). According to similar rice studies, screening at the reproductive stage for morphological traits like floret fertility is useful for identifying rice cultivars tolerant to salinity stress. This is because a higher floret fertility contributed to a high seed set and grain yield in tolerant genotypes, whereas a higher spikelet sterility led to a poor seed set and low grain yield in sensitive genotypes. This result might be a consequence of either the decrease in the viability of pollen or in the receptivity of the stigmatic surface; it might also be a consequence of both (Abdullah et al., 2001; Grattan et al. 2002; Khan and Abdullah, 2003; Zeng 2005; Sepaskhah

and Yousofi-Falakdehi, 2009). The 1000-grain weight was also significantly reduced under salinity conditions (Rao et al., 2008). Consequently, the salt-tolerant and high yielding rice cultivars for the Ergene Basin are Kral, Kırkpınar, Kros-424 and Durağan.

To solve salinity problems, mixing agricultural drainage water as well as low quality ground water with good quality river water in ratios to keep the salinity of the irrigation water below the threshold of the target crop is an acceptable practice. Alternating good quality water with drainage (saline water) is another management practice (Amer, 2010). Alternatively, a selection of crops or crop varieties capable of producing a profitable yield with saline wastewater (Qadir and Drechsel, 2010), must be found, as described in the present study. Previously, less attention has been given to the differences in salt tolerance between varieties of the same crop. This study addresses the gap in the data. Further studies are necessary to investigate the density of chlorophyll, proline, trehalose, and the antioxidant enzyme activities and anatomical properties of the cultivars.

### Conclusions

In this study, select morphometric and physiological parameters were examined to determine the most salt-tolerant and high yielding rice cultivars for use in Turkey's Ergene Basin. In 40 rice cultivars, the panicle fertility and 1000-grain weight were determined, and a cation analysis (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>) in the roots was performed. As a result of these experiments, the Durağan, Kros-424, Kıral and especially the Kırkpınar cultivars emerged as the most salttolerant and high yielding rice cultivars for the Ergene Basin.

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